

## Effect of Insulin on Ca<sup>2+</sup> Influx and Its Related Signal Pathways in Mouse Embryonic Stem Cells

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**Background :** Recent reports demonstrated that embryonic stem (ES) cells with pluripotency can differentiate into many cell types. Although [Ca<sup>2+</sup>]<sub>i</sub> plays an major role in the regulate of cellular functions, the effects of insulin and its related signal pathways on Ca<sup>2+</sup> uptake are not fully known in ES cells. Thus, we investigated the effect of insulin on Ca<sup>2+</sup> uptake in mouse embryonic stem cells.

**Methods :** <sup>45</sup>Ca<sup>2+</sup> uptake, western blotting analysis, cAMP assay, and Ips assay were performed in the mouse ES cells.

**Results :** We confirmed undifferentiated mouse ES cells by staining of alkaline phosphatase. Insulin significantly stimulated Ca<sup>2+</sup> uptake (24 hr, 5×10<sup>-10</sup> M). Its effect was blocked by genistein or herbimycin A (tyrosine kinase inhibitors). Insulin increased intracellular cAMP level and SQ 22536 (adenylate cyclase inhibitor), Rp<sup>8</sup>cAMP (cAMP analogue), or PKI (protein kinase A inhibitor) blocked the insulin-induced stimulation of Ca<sup>2+</sup> uptake. In addition, insulin rapidly increased inositol-1,4,5-triphosphate (Ips) generation compared to control. Insulin-induced stimulation of Ca<sup>2+</sup> uptake was blocked by nifedipine or methoxyverapamil (L-type Ca<sup>2+</sup> channel blockers). Staurosporine or bisindolylmaleimide I (PKC blockers) blocked insulin-induced increase of Ca<sup>2+</sup> uptake, suggesting involvement of PKC. In addition, wortmannin (PI-3 kinase inhibitor) blocked insulin-induced PKC activation but not SQ 22536. Effect of insulin on Ca<sup>2+</sup> uptake was blocked by SB 203580 (p38 MAPK inhibitor) but not PD 98059 (p44/42 MAPK inhibitor). Insulin increased p38 MAPK phosphorylation in western blotting analysis. In addition, genistein, PKI, and bisindolylmaleimide I blocked the phosphorylation of p38 MAPK by insulin.

**Conclusions :** Insulin increased Ca<sup>2+</sup> uptake via PKC, cAMP, and p38 MAPK signal pathways in mouse ES cells.